Chapter 17

Use of Spot Urine Sample Results in Physiologically Based Pharmacokinetic Modeling of Absorbed Malathion Doses in Humans

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Recently state health and regulatory agencies in California used a PB-PK model to estimate the absorbed doses of malathion in individuals allegedly exposed to aerial sprays during an urban pesticide application. Dose simulation in that study was performed on the results of single urine samples collected within 48 h of a potential exposure, and was based on a model validated with observed values from only a single volunteer. As a continuing effort another case study is presented in this chapter to validate the model with more human literature data. Results from this validation study showed that the time courses of the serial urinary malathion (metabolites) excretion presented in the literature were consistent with those simulated by the PB-PK model. When urine results collected from the literature cases at 8 - 12 h, 12 - 24 h, and 24 - 36 h or 24 - 48 h after initial exposure were postulated as spot samples, the majority (64/85) of the individual total absorbed doses simulated were within two-fold of their measured values, with no simulation doses exceeding three-fold. This validation study further showed that the accuracy would be improved considerably, if the simulation for each literature case were performed on two or more spot samples collected at different time points preferably within the first 24 or 36 h of exposure.

Biomarkers may be defined as properties that are capable of indicating that an individual has had exposure to a particular chemical (*1*). These indicators are measurable in biological media such as human cells or fluids, and are referred to as markers of internal dose. There are biological markers of other categories, such as markers of biologically effective dose and markers of disease (*2*). However, it is markers of internal dose that have given biological monitoring its long-standing place in the assessment of human exposure to pesticides. Of all the methods available for biological monitoring of exposure, the test most commonly used to assess the internal dose is the measurement of the chemical or its metabolite(s) in urine.

Despite its popularity in biological monitoring, urine analysis is not a test without limitations. Routine collection of 24-hour samples in human subjects is

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usually impractical. Urine analyses for pesticide exposure assessment are accordingly often necessarily performed on spot specimens. The excretion and other related disposition kinetics must then be used to interpret these analytical results. In addition, these results normally must be corrected for the total urine volume anticipated or excreted. One approach to a more effective interpretation of biomarker data based on spot samples is to simulate the internal dose through use of a physiologically based pharmacokinetic (PB-PK) model. This type of dose simulation can also simplify or even eliminate the task required for the correction of urine volume.

PB-PK models are designed to simulate the body as a series of tissue compartments, across which a chemical is absorbed, distributed, metabolized, and excreted in accord with pharmacokinetic rate laws and constants. Well-constructed PB-PK models can be powerful tools for interpretation of internal dose biomarker data, even of those data based on analysis of spot specimens. Such use was demonstrated in a 1994 study by Dong *et al.* (*3*), who used a PB-PK model to estimate the absorbed malathion doses in 11 adults and children allegedly exposed to aerial sprays during an urban pesticide application. Dose simulation in that study was performed on single urine samples collected within 48 h of a potential exposure, and was based on a PB-PK model validated with observed values from only a single volunteer. As a continuing effort, another case study is presented in this chapter to validate the PB-PK model with more human data now available in the literature.

In addition to validating the PB-PK model, the purpose of this chapter is to discuss the utility of this type of simulation in interpreting the data on internal dose markers that are analyzed especially on spot samples. It is also hoped that by presenting the results of this validation study in the literature, there will be a fuller appreciation of the absorbed doses of malathion simulated in the earlier 1994 case study.

Materials and Methods

PB-PK Simulation. In recent years numerous investigators have used PB-PK models to simulate tissue dose in the animal or human body, especially in those exposed to organic chemical vapors via inhalation. Over 15 references have been cited for work done in this subject area by Dong *et al.* (3), in which the basic structure of a PB-PK model for dermal exposure is included. The work done in that paper represents in effect a first collaborative effort by several state health and regulatory agencies in California to simulate absorbed doses of pesticides on spot urine samples from human volunteers. The PB-PK model used in the 1994 collaborative study was first constructed by the California Office of Environmental Health Hazard Assessment (*4,5*) to predict primarily urinary excretion of dermally applied malathion. The model was later modified by Dong *et al.* (*6*) to account more closely for the actual disposition and metabolic fate of malathion in humans. That modified model, together with the physiological and biochemical parameter values that were provided in the collaborative study, was used throughout this validation study. As in the collaborative study, all PB-PK simulations performed in this validation study were implemented with a microcomputer program written in BASICA (*7*).

Literature Data. Human data published on urinary excretion of malathion metabolites from dermal exposure are quite limited. All urine samples should be collected serially over a period of several days, if they are to be useful for dose simulation performed on spot samples. In addition, the dermal dose used should be at a level typical of human exposure and prepared in some aqueous or organic-based formulation. A review of the literature indicated that thus far only one study has been published to present data fulfilling these criteria for malathion. This is the work conducted in 1994 by Dary *et al.* (*8*). In their study, dermal absorption of neat malathion, a 50% emulsifiable concentrate (EC), and a 1% and 10% aqueous mixture of the 50% EC formulation was examined in 12 adult volunteers. The urine samples

from these human volunteers were collected over a 3-day period for recovery of radiolabeled malathion (mainly its metabolites). The total absorbed doses of malathion accumulated from these urinary radiorecoveries over the 72-hour period are listed in Table I below. These cumulative absorbed doses (in percent of their applied doses) were later used to validate the doses simulated from PB-PK modeling performed on spot urine samples.

In addition to the literature data published in 1994, there is one other earlier dermal absorption study available which provides serial urinary excretion of malathion over two consecutive 7-day periods. That study was published in 1983 by Wester *et al.* (*9*), whose interest was to contrast the percutaneous absorption of malathion from single dose applications with that after repeated administration. The data in the 1983 study were considered to be less desirable for the purpose of this validation study, in that the topical dose given to each of the 5 human volunteers was neat malathion at a level (5 mg/cm^2) which is much higher than those seen with typical exposure. Another drawback with the 1983 literature data is that there were no urine samples collected for 24 - 36 h after initial exposure. This interval is one of the crucial ones for the collection of spot samples (since from this time onward the excreted dose would be very low). Yet for completeness, the total absorbed doses calculated over the first 7 days (i.e., from single doses) for each of the 5 subjects in the 1983 study were also used in this validation study, and are included in Table I.

The third (and final) study of this type available in the literature is the classic work published in 1974 by Feldmann and Maibach (*10*). Their data were not used in this validation study since only the *group* mean values of urinary radiorecoveries taken over 6 volunteers were presented. The topical dose used in that classic work was also neat malathion, though at a much lower and hence a more typical dermal exposure level (4 $\mu g/cm^2$). Note that the intent of this validation study was to make use of the spot urine samples in estimating the total exposure for individuals rather than for groups of people. In general, it is relatively easier to more accurately estimate the total exposure for a group of people than for an individual.

There is still one other work not considered in this validation study. This is an earlier pilot study by Dong *et al.* (*6*), in which a comparison was made between dermal absorption of malathion simulated with PB-PK modeling for a single volunteer and those absorption values determined primarily in the 1983 and the 1974 studies cited above. Since the PB-PK model had been modified with much of the simulation results from that pilot study, it did not seem appropriate to *re*validate the simulation here with the same urinary data collected from that single volunteer. Another concern with the use of that set of urinary data is that the volunteer was an obese person with a body weight (~ 150 kg) twice that of an average adult. Body weight is related to blood flow, fat content, and some other physiological parameters included in the PB-PK model. Although there is a preferred formula that has been used to adjust for this body weight difference (*3-5*), the adjustment is far from perfect especially when an obese (*vs.* a lean heavyweight) person is considered. The rest of the human literature data included for comparison of malathion absorption in that pilot study were not presented in a time series (and hence could not be used here as spot samples for validating the PB-PK model).

Validation Procedures. For the purposes of this validation study, the PB-PK model was first applied to simulate the *serial* urinary excretion of malathion metabolites (including the small amount of unchanged malathion) observed in the 1983 and the 1994 literature cases. The endpoints used for simulation were the absorbed doses (in percent of the applied doses) accumulated to 72 h for cases in groups A through D and to the first 168 h for those in group E. The objective of this first phase was to assure that the time course of urinary excretion of malathion metabolites could be reproduced by the PB-PK model. The model was then used to simulate the total absorbed doses of malathion in these same literature cases under the assumption that only their spot urine samples collected at 8 - 12 h, 12 - 24 h, and 24 - 36 h or 24 - 48 h

Table I. Percent of the Applied Dose of ^{14}C-Malathion Recovered in the Urine of Human Volunteers at Selected Spot Intervals Following Topical Administration

Subject[a,b]	Dermal Dose mg/cm^2	8-12 h	12-24 h	24-36 h	24-48 h	Cumulative Abs. Dose[c]
		Percent of the Applied Dose Recovered				
A1	0.35	3.86	11.54	2.50		18.36
A2	0.92	0.55	1.06	0.58		2.45
A3	0.31	0.80	6.26	1.34		9.53
A4	2.03	1.35[d]	2.28	1.03		5.05
A5	0.43	0.41	1.72	0.50		3.10
A6	0.80		4.78[e]	0.55		5.62
B1	1.32	0.20	1.37	0.53		2.33
B2	1.20	0.74	2.14	1.12		4.24
B3	1.56	2.16	3.53	0.94		7.23
B4	0.57	2.19[d]	5.45	1.15		9.01
B5	0.39	1.30	5.42	1.62		9.04
B6	0.54	0.81[d]	0.47	1.11		2.57
C1	0.02	4.03	7.53	1.70		16.86
C2	0.03	4.22	10.36	3.10		19.59
C3	0.04	1.54	3.72	2.39		9.40
C4	0.03	20.50[d]	6.97	0.30		28.60
C5	0.03	4.67[d]	4.55	0.83		10.57
C6	0.03	0.53	8.94	1.02		11.93
D1	1.19	0.33	1.39	0.54		3.13
D2	1.20	1.18[d]	3.74	2.08		7.48
D3	1.18		2.83[e]	1.13		4.40
D4	1.19	1.25[d]	1.37	1.13		4.76
D5	1.17	0.33	2.25	1.95		5.27
D6	0.87	3.25[d]	4.85	2.28		10.99
E1	5.00	0.35	1.41		0.87	4.00
E2	5.00	0.56	1.88		0.34	3.97
E3	5.00	1.03	2.14		0.51	5.30
E4	5.00	0.25	0.37		0.34	2.07
E5	5.00	0.83	0.46		0.13	3.07

[a] subjects A1 through D6 were from the 1994 study by Dary *et al.* (*8*); after resting for two weeks, individuals in group A that were dosed with neat malathion were given again a 1.0% aqueous mixture as members of group C; individuals in group B receiving the 50% EC formulation were dosed again with the 10.0% aqueous mixture as members of group D after resting for two weeks.

[b] subjects E1 through E5 were from the 1983 study by Wester *et al.* (9); absorbed doses (from neat malathion) presented here for group E were *not* corrected for excretion by other routes (e.g., feces).

[c] cumulative absorbed dose in percent of the applied dose; accumulated to 72 h for groups A through D, and to the first (as from single doses only) 168 h for group E.

[d] at spot interval of 4 - 12 h, since no dose recovery was measured for 4 - 8 h and dose recovery for 8 - 12 h in this study subject was reported as dose accumulated to this interval.

[e] at spot interval of 4 - 24 h, since no dose recovery was measured for 4 - 8 h or for 8 - 12 h and dose recovery for 12 - 24 h in this study subject was reported as dose accumulated to this interval.

where applicable) after initial exposure were available. These time points were selected because they were considered to be reasonable for biological monitoring of exposure to malathion or to similar organophosphates. For example, for all the 11 adults and children in the 1994 collaborative study (*3*), the time lapse from first dermal contact until urine collection was reported to be within 12 h, 12 - 36 h, 24 - 36h, or 36 - 48 h.

In its simplest form, dose simulation with a PB-PK model would involve predictions of the *total* absorbed dose based upon a small (cumulative, temporal) amount of dose estimated from biomarker data. This task objective, together with the simulation procedures and the kinetic equations involved, was explicitly provided in the 1994 collaborative study. However, despite this provision it is important to note that urine contents measured in *spot* samples are normally not predictive of the amount of urinary excretion accumulated *to* that spot time point, unless there are no earlier voids given by the individual between that time and initial exposure. The cumulative percent of the applied dose recovered to that time point as quantified in spot urine samples, which is to be used as an endpoint for simulation of the total cumulative absorbed dose, is hence necessarily determined by some type of extrapolation.

One extrapolation method is to measure the urine concentration in the spot sample in some mass unit per volume of urine, and then multiply this concentration by the total urine volume that is expected to be voided between that time and initial exposure. This was the extrapolation method used in the 1994 collaborative study since its malathion metabolites were reported only in μg per liter of urine.

In the present validation study, a more accurate as well as more direct endpoint was used for dose simulation. Here urinary excretion *during* a spot interval was modeled by iterative simulation to the amount reported in the literature for that spot interval. The total cumulative absorbed dose of interest would then be read off the *entire* simulated serial urinary excretion. Note that even though only a portion of the urine content in a series is to be used as an endpoint for dose simulation, each run of the PB-PK modeling will simulate the urine and other tissue contents from time 0 to whatever time (e.g., at 72 h or 168 h) the analyst wishes the simulation to stop. The spot intervals used for validation with the 1994 literature data from Dary *et al.* (*8*) were 8 - 12 h, 12 - 24 h, and 24 - 36 h. As only cumulative percent of the applied dose recovered in urine was provided, the absorbed dose from the 1994 study for each postulated spot interval was determined by taking the difference between the absorbed dose of malathion accumulated to a given spot interval and the absorbed dose accumulated to the preceding interval in the series. Where the absorbed dose of malathion accumulated to the preceding interval was not available, the absorbed dose accumulated to the earlier interval was used instead. The spot interval targeted for validation would then be extended accordingly. These irregular, extended spot intervals are footnoted in Table I. The spot intervals used for validation with the 1983 literature data from Wester *et al.* (*9*) were 8 - 12 h, 12 - 24 h, and 24 - 48 h, since there were no urine samples collected specifically for the 24 - 36 h interval in that study. The absorbed dose presented in the 1983 study for each interval in the series, while also in percent of the applied dose, was *non*cumulative.

Results and Discussion

Results of this validation study showed that the time courses of the serial malathion metabolites (including the very small amount of unchanged malathion) present in human urine reported in the literature were reproducible by the PB-PK model. These findings were also found to be quite consistent with those observed earlier in a pilot study on urine results from a single volunteer (*6*). The time courses of malathion metabolites simulated in this validation study for subjects A1, B1, C1, D1, and E1, together with their observed serial urinary excretion, are summarized in Figures 1 through 5, respectively.

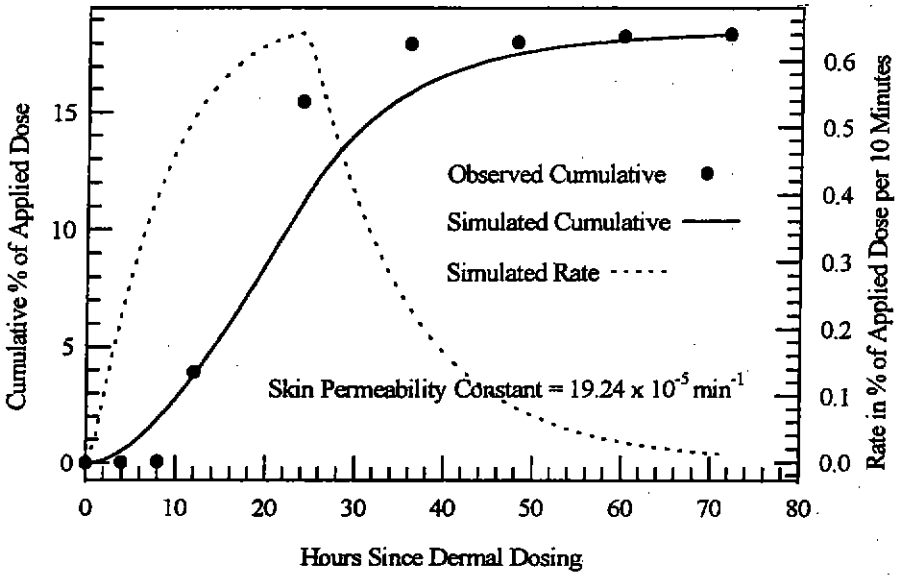

Figure 1. Excretion of urinary malathion (mainly its metabolites) observed *versus* simulated for subject A1 in Dary *et al.* (*8*).

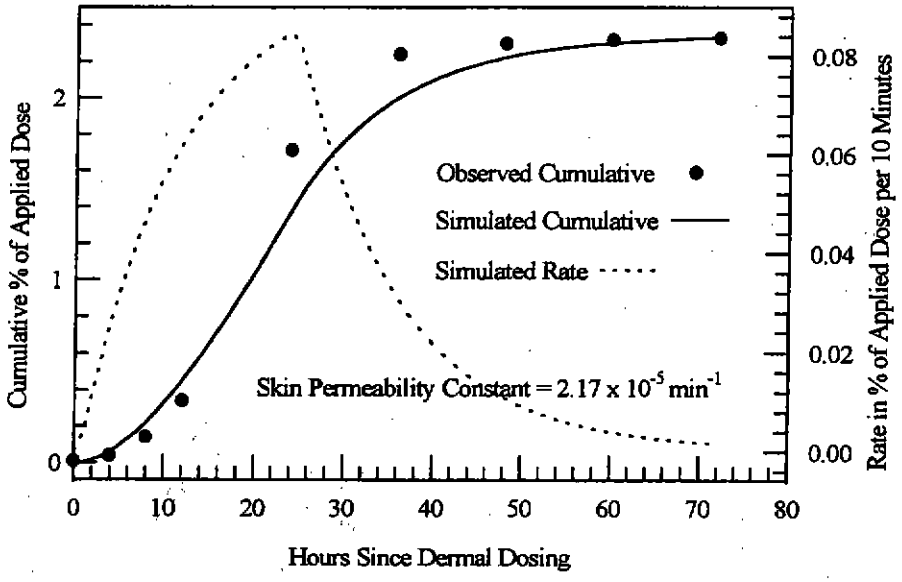

Figure 2. Excretion of urinary malathion (mainly its metabolites) observed *versus* simulated for subject B1 in Dary *et al.* (*8*).

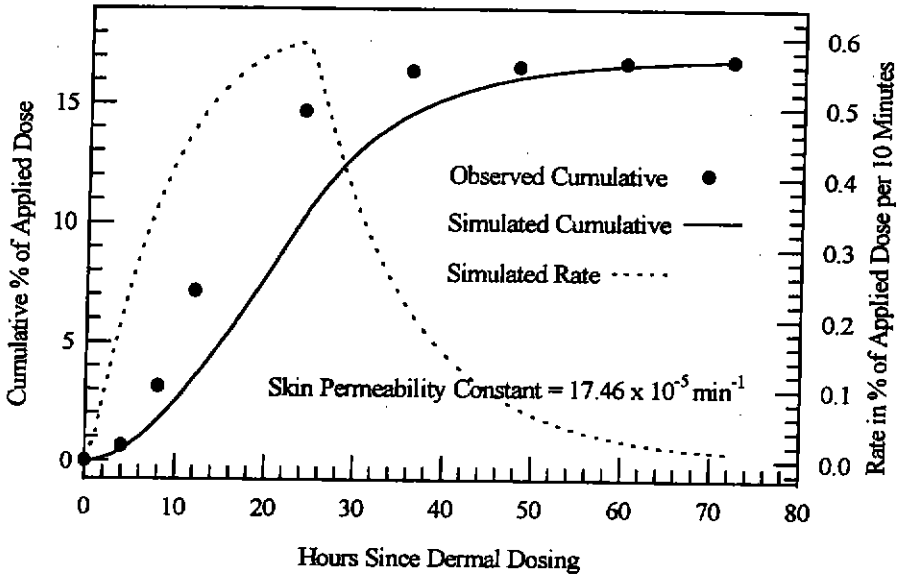

Figure 3. Excretion of urinary malathion (mainly its metabolites) observed *versus* simulated for subject C1 in Dary *et al.* (*8*).

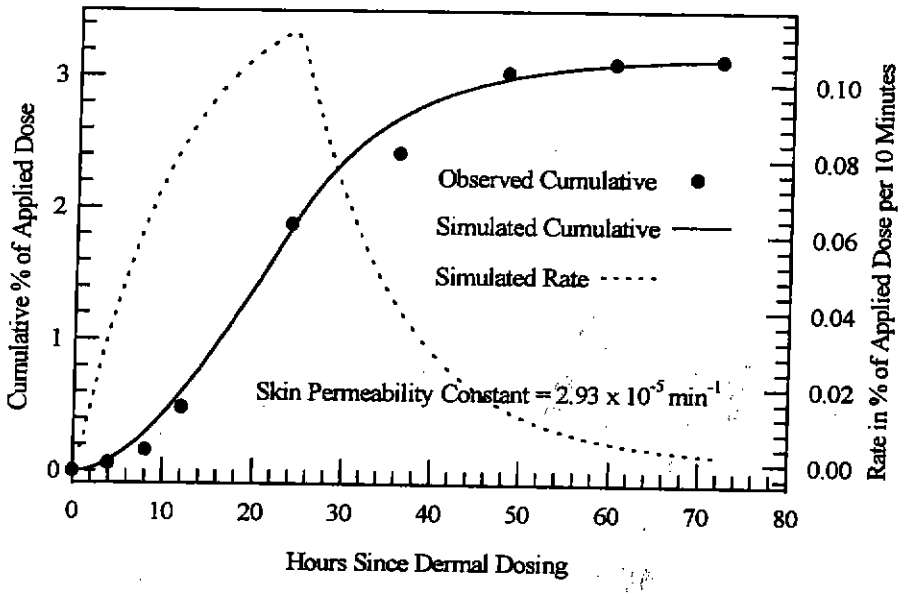

Figure 4. Excretion of urinary malathion (mainly its metabolites) observed *versus* simulated for subject D1 in Dary *et al.* (*8*).

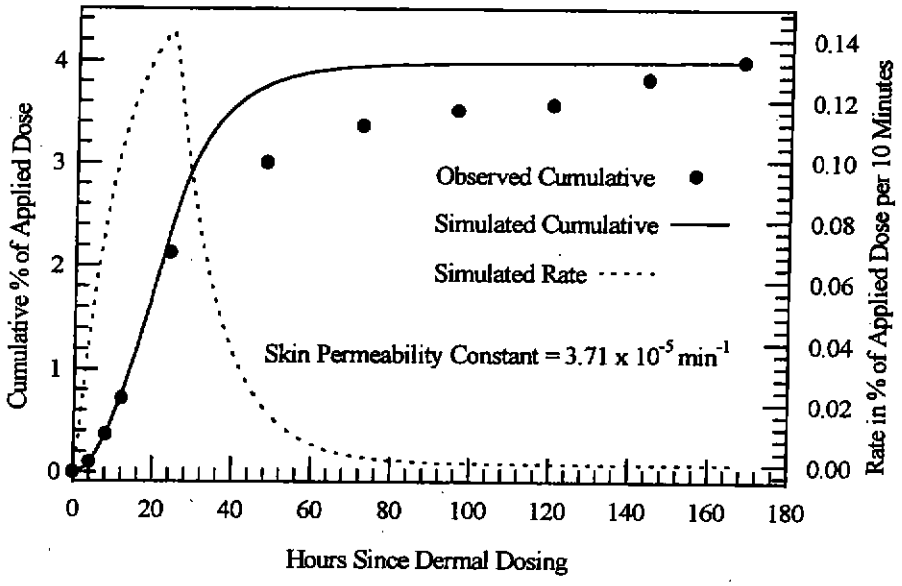

Figure 5. Excretion of urinary malathion (mainly its metabolites) observed *versus* simulated for subject E1 in Wester *et al.* (*9*).

Figures 1 through 5 exemplify the high degree of reproducibility achieved by the PB-PK model, especially in light of the fact that a physiological and not a physical model is considered here. The observed values presented in these figures were from the human studies by Dary *et al.* (*8*) and by Wester *et al.* (*9*), who like other investigators did not have full control of the human experimental situation (e.g., subjects were not cannulated, their dietary intake not assessed, their fluid intake not controlled, etc.). Insofar as there was no assurance for the *absolute* accuracy of *each* of these observed values, it did not seem worthwhile to perform any goodness-of-fit test for the simulated time series.

Also included in Figures 1 through 5 are the excretion rates (per 10 minutes) simulated at various time intervals for these same individuals. As shown (dotted lines), the excretion rates for these subjects (and for the rest of those not shown) peaked at approximately 18 to 24 h after initial exposure. The peak hours of excretion rates simulated in this validation study were found to be in good agreement with those observed in the 1983 study by Wester *et al.* (*9*) and in the 1994 study by Dary *et al.* (*8*). These peak hours seen in the two literature studies (and in this simulation study as well) were approximately 2 to 3 times greater than those simulated earlier in the 1994 collaborative study by Dong *et al.* (*3*). The reason for this difference in peak hours is that the dermal dose applied to the volunteers in the literature studies was left on their skin for a period of 24 h, whereas the subjects in the collaborative study were assumed to be exposed for approximately 8 h before they took a bath or shower. Insofar as duration is concerned, the 8-hour exposure scenario seems to be more realistic (or more frequently encountered in real life situations).

Figure 1 indicates that there might be a few hours of short lag before the dermal absorption of malathion could take place in subject A1. Similar indication was seen earlier in the single volunteer in the pilot study (*6*), but not particularly in the

other literature cases considered in this validation study. As footnoted in Table I, subjects in study group A that were dosed with neat malathion were later given again a 1.0% aqueous mixture as members of study group C. However, it is not clear from the information available whether subject A1 and subject C1 were the same individual. If they were, then the short lag seen in Figure 1 (i.e., subject A1) and not in Figure 3 (i.e., subject C1) might have been due at least in part to the dilution of the malathion used in that experiment. The emotional, nutritional, and physiological state, etc. at the time the study subject was exposed to the malathion dilution might also have played an important role.

The variable skin permeability constants shown in Figures 1 through 5 were the only model input parameter that needed to be adjusted until the model prediction of malathion (and its metabolites) matched the amount estimated from the biomarker data (for the spot interval in question). This adjustment should not have violated the underlying principles of PB-PK modeling averred by McDougal *et al.* (*11*) and by Knaak *et al.* (*12*). According to these investigators, the total absorbed dose from dermal exposure is simply a function of three input parameters. These input parameters are the skin permeability constant P, the total applied (topical) dose D, and the permeation time T. In this validation study, the input parameters were further reduced to T (which was fixed at 24 h) and P because the absorbed dose was calculated in percent of the applied dose. (In the 1994 collaborative study, the parameter D was written as the product of the total exposed surface area A and the exposure concentration C.) As described elsewhere (*3-7,13*), the PB-PK model consists of a series of differential equations which collectively contain numerous biochemical and physiological variables and constants, such as blood flow rate to a particular organ, the organ volume, and Michaelis constant. However, for purposes of this dose simulation, these (other) variables and constants are not considered as the true *input* parameters in that they are known or at least fixed (regardless of the amount of dermal dose to be absorbed).

The total internal doses of malathion simulated from the various spot urine samples are summarized in Table II. This table shows that none of the total absorbed malathion doses simulated for the 17 volunteers was more than 3 times its measured value. The majority (64/85 = 75%) of the simulation doses were within two-fold of their measured values. A few (6/85 = 7%) simulation doses were found to be unacceptably (≥ three-fold) lower than their measured values, however; these were the absorbed doses simulated from spot samples collected at 24 - 36 h or 24 - 48 h. The averages taken over the three absorbed doses simulated (from the three spot samples) for each study subject were found to be highly comparable to the measured values. As shown in Table II, the ratios of these averages to the observed (measured) values ranged from 0.7 to 1.4, with an arithmetic mean of 1.1.

By and large, the absorbed doses simulated on spot samples collected between 12 and 24 h (after initial exposure) appeared to be most comparable to their literature values. This group of simulation doses had an arithmetic mean ratio (to the measured values) of 1.2. This finding is not surprising at all, given that the excretion rates for these subjects were seen to peak at this interval. The dose simulation theoretically should be most accurate on spot samples collected at this interval, in that sensitivity and specificity would become less an issue at this interval in quantifying the urine content from the samples collected. Despite the scarcity of data, there appears to be some indication from Table II that neither chemical formulation nor exposure level will have a significant effect on the accuracy of dose simulation.

As pointed out in the collaborative study by Dong *et al.* (*3*), the PB-PK model used here specified that slightly over 75% of an absorbed dose of malathion would be recovered in urine with another 20% recovered in feces. The urinary recovery simulated with this PB-PK model was thus approximately 15 to 20% lower than those dose recoveries observed experimentally by Feldmann and Maibach (*10*) and by Ross *et al.* (*14*). This difference in urinary excretion for malathion metabolites (including

Table II. Total Percent of Absorbed Dose of Malathion Observed in the Urine of Human Volunteers *versus* Simulated, based on Selected Spot Intervals Following Topical Administration

Subject[a]	Observed Dose[b]	8-12 h	12-24 h	24-36 h	24-48 h	Average[c]
		Total Percent of Absorbed Dose Simulated from Spot Sample[d]				
A1	18.4	34.3 (1.9)	29.1 (1.6)	9.4 (0.5)		24.3 (1.3)
A2	2.5	5.5 (2.3)	2.7 (1.1)	2.1 (0.9)		3.5 (1.4)
A3	9.5	8.0 (0.8)	15.8 (1.7)	5.0 (0.5)		9.6 (1.0)
A4	5.1	7.9 (1.6)[e]	5.8 (1.2)	3.8 (0.8)		5.8 (1.2)
A5	3.1	4.2 (1.3)	4.4 (1.4)	1.8 (0.6)		3.5 (1.1)
A6	5.6		8.4 (1.5)[f]	2.0 (0.4)		5.2 (0.9)
B1	2.3	2.0 (0.9)	3.5 (1.5)	1.9 (0.8)		2.5 (1.1)
B2	4.2	7.4 (1.7)	5.4 (1.3)	4.2 (1.0)		5.7 (1.3)
B3	7.2	20.5 (2.8)	8.9 (1.2)	3.5 (0.5)		11.0 (1.5)
B4	9.0	12.5 (1.4)[e]	13.8 (1.5)	4.3 (0.5)		10.2 (1.1)
B5	9.0	12.7 (1.4)	13.7 (1.5)	6.0 (0.7)		10.8 (1.2)
B6	2.6	4.8 (1.9)[e]	1.2 (0.5)	4.1 (1.6)		3.4 (1.3)
C1	16.9	35.5 (2.1)	19.0 (1.1)	6.3 (0.4)		20.3 (1.2)
C2	19.6	36.9 (1.9)	26.2 (1.3)	11.7 (0.6)		24.9 (1.3)
C3	9.4	14.9 (1.6)	9.4 (1.0)	9.0 (1.0)		11.1 (1.2)
C4	28.6	70.5 (2.5)[e]	17.6 (0.6)	1.1 (0.04)		29.8 (1.0)
C5	10.6	25.1 (2.4)[e]	11.5 (1.1)	3.1 (0.3)		13.2 (1.3)
C6	11.9	5.3 (0.5)	22.6 (1.9)	3.8 (0.3)		10.6 (0.9)
D1	3.1	3.3 (1.1)	3.5 (1.1)	2.0 (0.6)		3.0 (0.9)
D2	7.5	6.9 (0.9)[e]	9.5 (1.3)	7.8 (1.0)		8.1 (1.1)
D3	4.4		5.0 (1.1)[f]	4.2 (1.0)		4.6 (1.1)
D4	4.8	7.3 (1.5)[e]	3.5 (0.7)	4.2 (0.9)		5.0 (1.1)
D5	5.3	3.3 (0.6)	5.7 (1.1)	7.3 (1.4)		5.4 (1.0)
D6	11.0	18.1 (1.7)[e]	12.3 (1.1)	8.5 (0.8)		13.0 (1.2)
E1	4.0	3.6 (0.9)	3.6 (0.9)		2.4 (0.6)	3.2 (0.8)
E2	4.0	5.7 (1.4)	4.8 (1.2)		0.9 (0.2)	3.8 (1.0)
E3	5.3	10.3 (1.9)	5.5 (1.0)		1.4 (0.3)	5.7 (1.1)
E4	2.1	2.6 (1.3)	1.0 (0.5)		0.9 (0.5)	1.5 (0.7)
E5	3.1	8.4 (2.7)	1.2 (0.4)		0.4 (0.1)	3.3 (1.1)
mean	*7.9*	*14.0 (1.6)*	*9.5 (1.2)*	*4.9 (0.7)*	*1.2 (0.4)*	*9.0 (1.1)*

[a] same individuals as those listed in Table I; groups A through D were from Dary *et al.* (*8*) and group E, from Wester *et al.* (*9*).

[b] same (rounded off to one decimal place) as those listed under cumulative absorbed dose in Table I.

[c] arithmetic mean taken over the total absorbed doses of malathion simulated from the three selected spot urine samples.

[d] in parentheses is the ratio of the total absorbed dose simulated from the selected spot sample (or of the average simulated dose) to the observed value.

[e] simulated for spot interval of 4 - 12 h (*see* footnote *d* in Table I for explanation).

[f] simulated for spot interval of 4 - 24 h (*see* footnote *e* in Table I for explanation).

the small amount of unchanged malathion) should have caused a slight oversimulation of the total absorbed doses from the PB-PK modeling. Underlying this dose overestimation is the fact that in order for the same amount of urinary output to be equivalent to the amount estimated from biomarker data, a greater absorbed dose has to be simulated or called for if the presumed ratio of urinary excretion to excretion by other routes (e.g., feces) is smaller.

For the ratio of urinary excretion to excretion by other routes in the model to be consistent with that observed experimentally, an adjustment should be made in the model for the physiological and biochemical parameter values, including the fecal and the urinary constants, that were assumed especially for the gastrointestinal tract and the kidney compartments. Thus far the PB-PK model has not been revised to increase this excretion ratio. The main reason for not revising the PB-PK model in this regard is that this excretion ratio could very well be sex-, body weight (fat)-, or age-related. More experimental data are required in order to confirm this speculation. After all, the greater excretion ratio observed by Feldmann and Maibach (*10*) was based on 6 human volunteers whose individual physiological characteristics were not given. Also, the urinary excretion of these 6 subjects from intravenous administration (to account for excretion via urine versus other routes) was reported in the form of a group mean, with a large variation coefficient of 10.8% (relative to the 15 - 20% difference seen in excretion ratio). The greater excretion ratio observed in Ross *et al.* (*14*) likewise must be interpreted with care, as that study for human clearance of malathion involved only a single volunteer.

The human subjects in this validation study were assumed to have a typical body weight of 70 kg since their individual physiological characteristics were not given. Some of the physiological and biochemical parameter values used in a (this) PB-PK model are related closely to body weight. Accordingly, the accuracy of dose simulation might have been improved somewhat if these parameter values used in the PB-PK model were tailored more closely to those actually governing the excretion and disposition kinetics in the literature cases. Sensitivity analyses performed in the 1994 collaborative study (*3*) indicated that model predictions were most affected (up to as much as two-fold) by the skin-blood partition coefficient, which tends to be highly chemical-specific and is related closely to skin permeability. Although many other input parameters (such as tissue volume and tissue perfusion rate) are also sensitive to body weight, they tend to be affected *proportionally* as well as *unselectively* by body weight and hence as a group have less effect on dose simulation.

Overall, the absorbed doses simulated in this validation study were much more comparable to the literature values than were those doses simulated earlier in a pilot trial published recently (*13*). Only literature data published in the 1994 study by Dary *et al.* (*8*) were attempted in that trial study. The highest doses simulated for the 12 human subjects in that trial were up to 5 or 6 times greater than the measured values. Those comparatively poorer results were mainly due to the use of a different endpoint for dose simulation. In that trial study, model predictions of the urinary excretion of malathion accumulated *up to* (instead of *during*) the spot interval were simulated. In addition, the literature cumulative dose value for each of the spot samples was approximated by *multiplying* the total time lapsed since initial exposure *by* the hourly rate of excretion determined for that interval. (Note that in real life situations where spot specimens are used, there will not be any actual value known for total urinary excretion *up to* the spot interval in question.) This type of approximation should yield poorer simulation results since, as shown in Figures 1 through 5, excretion rates for malathion do vary over time. That method of approximation was nonetheless used in the trial study to offer an alternative for a situation where the urine content in spot samples is reported in some mass unit per volume of urine. An actual case in point is again the 1994 collaborative study by Dong *et al.* (*3*), in which the urine contents of malathion metabolites for the 11 adults and children were available only in μg per liter of urine.

Conclusion

The results in this validation study suggest that PB-PK simulation can be performed on spot urine samples to predict the total absorbed dose of malathion in humans, with an accuracy likely to be well within a few fold of actual (dermal) exposure. The best spot samples appear to be those collected around the peak hours of urinary excretion. There appears to be some indication that neither chemical formulation nor exposure level will have a significant effect on the accuracy of dose simulation. The data also show that the accuracy could be improved considerably, if the dose simulation for each study subject were performed on two or more spot specimens collected at different time points preferably within the first 24 or 36 h of exposure to malathion.

It was also found that spot urine samples would be most useful in PB-PK modeling of the total absorbed doses (of malathion) if the urine content were measured as the total amount accumulated during a given spot interval, instead of in some mass unit per volume of urine. It is further recommended that for each study subject, the values of some basic physiological parameters such as body weight, sex, and age be supplied along with the spot urine sample(s). Above all, the spot *interval* (especially relative to initial exposure) should be measured or reported as accurately as feasible.

Disclaimer

No official endorsement of particular computer software by the California Department of Pesticide Regulation or by the University of California is intended or implied; the opinions expressed are those of the authors and not necessarily those of the Department, of any other California State agency, or of the University of California.

Literature Cited

1. Mendelsohn, M. L. In *Biomarkers and Occupational Health*; Mendelsohn, M. L.; Peeters, J. P.; Normandy, M. J., Eds.; Joseph Henry Press: Washington, D. C., 1995; pp *iii-iv*.
2. Vine, M. F. In *Environmental Epidemiology - Effects of Environmental Chemicals on Human Health*; Draper, W. M., Ed.; Advances in Chemistry Series 241; American Chemical Society: Washington, D. C., 1994; pp 105-120.
3. Dong, M. H; Draper, W. M.; Papanek, P. J., Jr.; Ross, J. H.; Woloshin, K. A.; Stephens, R. D. In *Environmental Epidemiology - Effects of Environmental Chemicals on Human Health*; Draper, W. M., Ed.; Advances in Chemistry Series 241; American Chemical Society: Washington, D. C., 1994; pp 189-208.
4. *Health Risk Assessment of Aerial Application of Malathion-Bait*; California Environmental Protection Agency, Office of Environmental Health Hazard Assessment: Sacramento, CA (Reprints available from Copies Unlimited, 5904, Sunset Boulevard, Los Angeles, CA 90028.), 1991.
5. Rabovsky, J.; Brown, J. P. *J. Occup. Med. Toxicol.* **1993**, *2*, 131-168.
6. Dong, M. H.; Ross, J. H.; Thongsinthusak, T.; Sanborn, J. R.; Wang, R. G. M. *Physiologically-Based Pharmacokinetic (PB-PK) Modeling for Dermal Absorption of Pesticide (Malathion) in Man*; Worker Health and Safety Branch, California Department of Pesticide Regulation: Sacramento, CA, 1993; Technical Report HS-1678 (abstract published in *The Toxicologist*, **1993**, *13*, 355).
7. Dong, M. H. *Comp. Meth. Prog. Biomed.* **1994**, *45*, 213-221.
8. Dary, C. C.; Blancato, J. N.; Castles, M.; Reddy, V.; Cannon, M.; Saleh, M. A.; Cash, G. G. In *Biomarkers of Human Exposure to Pesticides*; Saleh, M. A.; Blancato, J. N.; Nauman, C. H., Eds.; American Chemical Society Symposium Series No. 542; American Chemical Society: Washington, D. C., 1994; pp 231-263.

9. Wester, R. C.; Maibach, H. I.; Bucks, D. A. W.; Guy, R. H. *Toxicol. Appl. Pharmacol.* **1983**, *68*, 116-119.
10. Feldmann, R. J.; Maibach, H. I. *Toxicol. Appl. Pharmacol.* **1974**, *28*, 126-132.
11. McDougal, J. N.; Jepson, G. W.; Clewell, H. J., III; Gargas, M. L.; Andersen, M. E. *Fund. Appl. Toxicol.* **1990**, *14*, 299-308.
12. Knaak, J. B.; Al-Bayati, M. A.; Raabe, O. G. In *Health Risk Assessment - Dermal and Inhalation Exposure and Absorption of Toxicants*; Wang, R. G. M.; Knaak, J. B.; Maibach, H. I., Eds.; CRC: Boca Raton, FL, 1993, pp 3-29.
13. Dong, M. H., Thongsinthusak, T.; Ross, J. H.; Krieger, R. I. *Validation of a Physiologically Based Pharmacokinetic (PB-PK) Model Used to Simulate Absorbed Malathion Doses in Humans*; Worker Health and Safety Branch, California Department of Pesticide Regulation: Sacramento, CA, 1995; Technical Report HS-1718 (presented as a poster paper at the 209th American Chemical Society Annual National Meeting in Anaheim, California, April 2 - 6, 1995).
14. Ross, J. H.; Thongsinthusak, T.; Krieger, R. I.; Frederickson, S.; Fong, H. R.; Taylor, S.; Begum, S.; Dong, M. H. *Human Clearance of Malathion*. Worker Health and Safety Branch, California Department of Pesticide Regulation: Sacramento, CA, 1991; Technical Report HS-1617 (abstract published in *The Toxicologist*, **1991**, *11*, 160).

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